PCR-Ribotype variability of *Clostridium difficile* strains from the patients with hospital-acquired *C. difficile* infections (HACDI), community-acquired CDI (CACDI), toxigenic colonization and non-toxigenic colonization

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ABSTRACTS

Background and Aim: According to the pathophysiology of CDI, the strains from the patients with HACDI and CD colonization would be similar in an institution. However, few data presented direct comparison of ribotype distribution of the CD strains from CDI and colonization in an institution. In order to help understanding the epidemiology of CDI in a hospital, we compared the PCR ribotypes of CD strains from HACDI and toxigenic colonization as well as from CACDI and non-toxigeneic colonization using the stool samples submitted for CD cultures in an institution during 3 years.

Methods: All CD strains from stool submitted for CD cultures in Hanyang University Hospital during the year of 2009, 2012 and 2014 were included. Detection of toxin genes using multiplex PCR and PCR ribotyping were performed as described previously, and restrospective chart review was performed as well. According to the carriage of toxin genes and presence of disease, the enrolled patients were categorized into 4 epidemiological groups of CACDI, HACDI, toxigenic colonization and non-toxigenic colonization.

Results: During the 3 years, 751 CD strains were identified from 751 patients. Among the 751 patients, 20 were CACDI, 455 HACDI, 142 toxigenic carriers and 134 non-toxigenic carriers. Common RTs were RT017 (25%), RT112 (20%), RT012 (10%), RT015 (10%), RT014 (5%), RT018 (5%), unkown ribotype 40 (UNK40) (10%), and UNK46 (5%) in CACDI; RT018 (38.2%), RT017 (13.9%), RT002 (8.1%), RT015 (7.5%), RT001 (7%), RT014 (3%), RT112 (2.9%), RT293 (2.6%), and more in HACDI; RT018 (30.3%), RT017 (12.7%), RT015 (12%), RT012 (10.6%), RT112 (7%), RT001 (3.5%), RT002 (2.8%), and more in toxigenic colonization; all unkown ribotypes except one RT012 and two RT018 in non-toxigenic colonization. Comparing the diversity of ribotypes among epidemiological groups, CACDI showed the highest and HA-CDI the lowest, and non-toxigenic and toxigenic colonization in-between values. Although most strains from non-toxigenic colonization were unknown ribotypes, they were also clustered (maximum 19 strains showed the same PCR ribotypes).

Conclusions: CD strains from CACDI, HACDI, toxigenic colonization and non-toxigenic colonization varied in an institution during the same year. CD strains from CACDI were the most diverse and those from HACDI showed a least diversity.

MATERIALS & METHODS

❖ Difinition of clinical diagnosis

- *C. difficile* infection (CDI): Patients who showed any positive results listed below

  • Laboratory test; *C. difficile* toxin A&B assay or cultured organisms with tcdA or tcdB confirmed by PCR

  • Image study; pseudomembrane by endoscopy or histology

- Hospital-acquired CDI (HACDI):

  • developed diarrhea at least 72 h after hospitalization

  • within 2 months of the last discharge provided that they were not residents in a long-term facility

- Community-acquired CDI (CACDI): the case did not meet the definition of HA CDI

- Toxigenic colonization: patients with *C. difficile* containing positive toxin genes in stool or positive results of toxin

  assay A&B but whose stool characters did not meet the criteria of diarrhea

- Non-toxigenic colonization: patients *with C. difficile* without toxin genes in stool

❖Study period and patients: All CD strains from stool submitted for CD cultures in Hanyang University Hospital during the year of 2009, 2012 and 2014

❖Multiplex PCR ; with cultured isolates, identified *tcd*A, *tcd*B, *cdt*A and *cdt*B

❖PCR ribotyping ; compared with reference strains (PCR-ribotype 017, 027, and strains from the ECDC-Brazier collection)

RESULTS

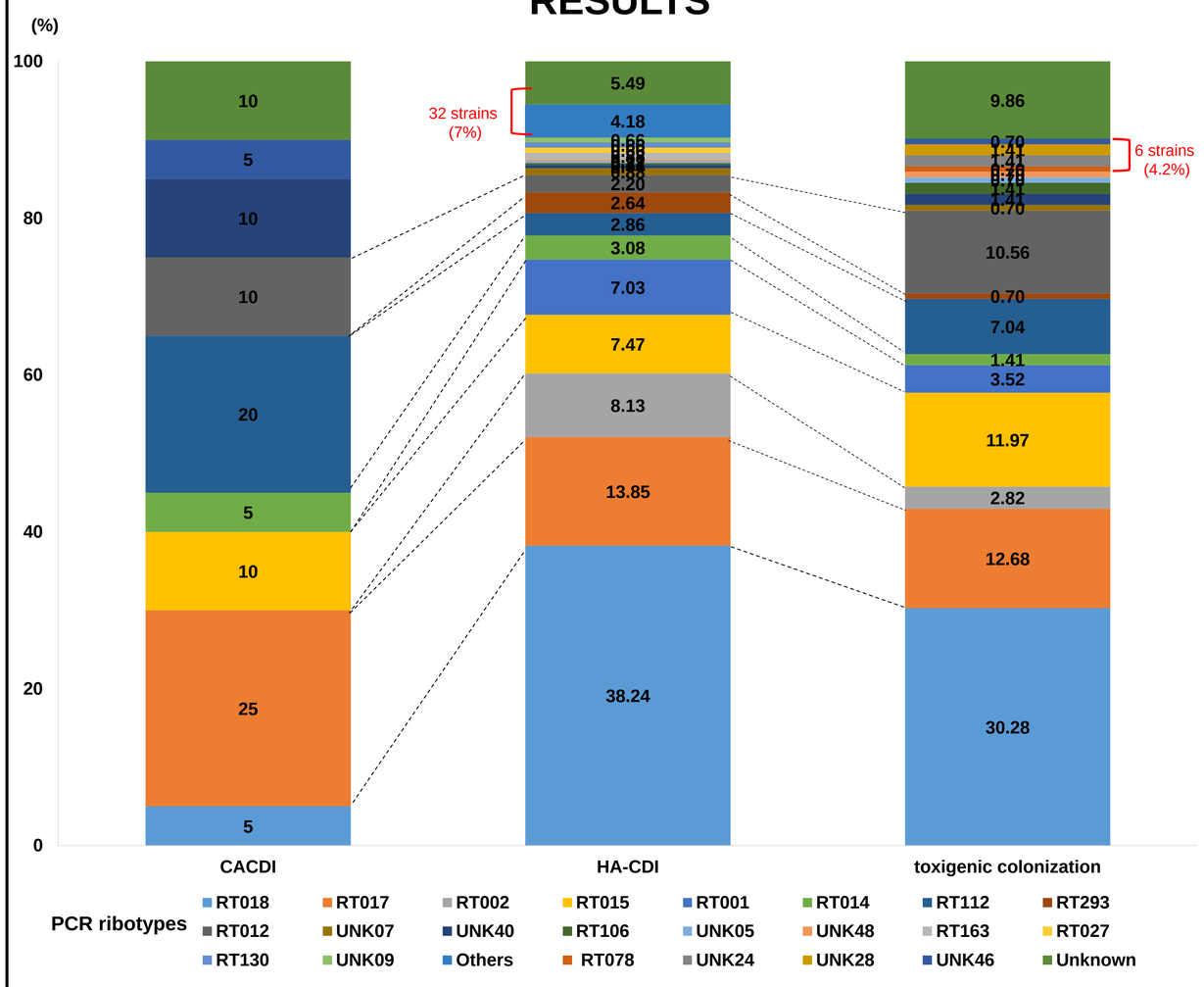

Figure 1. Distribution of *Clostridium difficile* according to clinical diagnosis. Among 751 strains, 20 (2.7%) were community-acquired *C. difficile* infections (CACDI), 455 (60.6%) hospital-acquired CDI (HACDI), 142 (18.9%) toxigenic colonization and 134 (17.8%) non-toxigenic colonization. Seventeen RTs with 32 strains (7%) from HACDI were not identified in toxigenic colonized patients, and 4 RTs with 6 strains (4.2%) from toxigenic colonization did not make infections.

Table 1. Composition of PCR ribotypes in 751 *Clostridium difficile* isolates by epidemiologic category

	CACDI	HA-CDI	toxigenic colonization	colonization
Number of PCR ribotypes	10	56	32	48
Total isolates	20	455	142	134
Diversity index, [a]%	50	12.31	22.54	35.82

[a] The diversity index of PCR ribotypes is the number of unique PCR ribotypes divided by number of isolates in eacht epidemiologic category

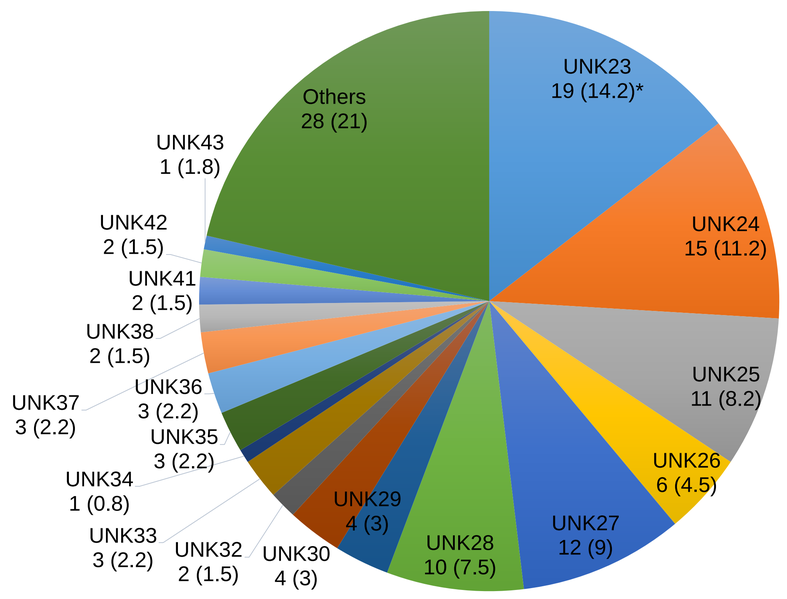

Figure 2. Strains from non-toxigenic colonization were also clustered according to the PCR ribotypes (maximum 19 strains) in 134 non-toxigenic *Clostridium difficile* colonization. * Number of strains (%)

Table 2. Clustering of the strains from non-toxigenic *Clostridium difficile* colonization

	2009	2012	2014	total
UNK23	16	3		19
UNK24	4	5	6	15
UNK25	2	6	3	11
UNK26	3	3		6
UNK27		7	5	12
UNK28	1	7	2	10
UNK29	2		2	4
UNK30			4	4